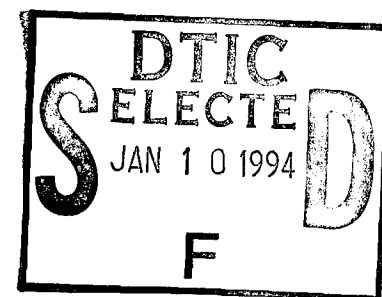


19950109 150

# Decreased Pulmonary Damage in Primates with Inhalation Injury Treated with High-Frequency Ventilation

William G. Cioffi, M.D.,\* Robert A. deLemos, M.D.,† Jacqueline J. Coalson, Ph.D.,‡  
Dale A. Gerstmann, M.D.,† and Basil A. Pruitt, Jr., M.D.\*

*From the U. S. Army Institute of Surgical Research,\* Fort Sam Houston, and the Southwest Research Institute,† and the Department of Pathology,‡ University of Texas at San Antonio Health Science Center, San Antonio, Texas*



## Objective

This study compared two forms of high-frequency ventilation (HFV) with conventional volume ventilation (CON) in a primate model of inhalation injury to determine whether ventilatory mode was a determinant of pulmonary damage.

## Summary Background Data

The authors previously reported that the prophylactic use of high-frequency flow interruption in patients with bronchoscopically diagnosed inhalation injury requiring mechanical ventilatory support resulted in a significant decrement in mortality. They hypothesized that a reduction in ventilatory mode induced pulmonary damage was in part responsible for their clinical results.

## Methods

Fifteen adult baboons were randomized to one of three ventilatory modes (CON, high-frequency flow interruption [HFFI], or high-frequency oscillatory ventilation [HFO]) after moderate smoke injury. Ventilatory support was tailored to the same physiologic endpoints. After 7 days, the animals were killed and pulmonary pathologic changes were scored and compared. Repetitive physiologic and biochemical data were compared using analysis of variance for repeated measures.

## Results

Physiologic endpoints were achieved in CON and HFFI, but not in HFO. Hemodynamic variables did not differ between CON and HFFI. The barotrauma index was greater in CON compared to HFFI ( $p < 0.05$ ), despite similar  $PO_2$ ,  $FIO_2$ , AA gradient, and  $PCO_2$ . Animals treated with HFFI had significantly less parenchymal damage than those treated with CON ( $p = 0.03$ ) or HFO ( $p = 0.0008$ ).

## Conclusions

The prophylactic use of HFFI led to a significant decrement in ventilatory mode induced pulmonary damage and offers an explanation for the decreased mortality in inhalation injury patients treated with HFFI.

Smoke inhalation leads to a complex sequence of pulmonary and pathophysiologic events that contribute to a high morbidity and mortality when combined with thermal injury.<sup>1,2</sup> While the volume and composition of the inhaled material clearly influence the severity of the pulmonary manifestations of inhalation injury, other data suggest that the mode of ventilatory support may also affect the severity of the disease process.<sup>3</sup>

The term "high-frequency ventilation" (HFV) describes a group of ventilatory techniques that use low tidal volumes at supraphysiologic respiratory frequencies. When used with appropriate ventilation strategies, HFV can maintain the lung at a given mean volume/pressure with less excursions above and below the mean than can be accomplished with conventional mechanical ventilation. This, in theory, should lessen the risk of ventilator-induced lung injury.

The use of high-frequency oscillatory ventilation (HFO) at high mean airway pressures has been shown to alter markedly the progression of both adult respiratory distress syndrome (ARDS) and hyaline membrane disease in experimental models. In rabbits, use of HFO prevented edema, hyaline membrane formation, and the loss of membrane compliance seen in surfactant depleted animals treated with conventional ventilation (CON) after saline lung lavage.<sup>4</sup> Even more dramatic findings have been documented in surfactant-deficient premature baboons treated with HFO. The initiation of HFO before the first breath prevented development of the pathologic, physiologic, and morphologic features of hyaline membrane disease when compared to animals of comparable gestational age treated with conventional positive pressure ventilation.<sup>5,6</sup> The development of hyaline membrane disease in this model was associated with increased levels of platelet activating factor-like activity in the lung lavage, while no increase in such activity was seen in HFO-treated animals. These data suggest that in the face of surfactant deficiency, conventional tidal ventilation leads to epithelial injury, mediator release, and increased parenchymal injury.

We have reported that the prophylactic use of high-frequency flow interruption (HFFI) was effective in reducing mortality in patients with inhalation injury.<sup>3</sup> The mechanism responsible for this decrement in mortality is not known. HFFI has been shown to be of no benefit in the prevention of ARDS in adults.<sup>7</sup> In some studies,

HFO has been efficacious in the management of infants with diffuse alveolar disease, although other studies have shown no dramatic improvement.<sup>8</sup> Studies concerning the prophylactic use of HFO in adult humans are lacking. This study compared the effects of two forms of high-frequency ventilation with conventional positive pressure ventilation in a primate model of moderate inhalation injury in an attempt to determine whether reduction in ventilator-induced lung injury was in part responsible for the clinical success.

## METHOD AND MATERIALS

### High-Frequency Ventilators

HFFI is a form of HFV in which inhalation is active and exhalation is passive.<sup>9</sup> Tidal volumes less than dead space volume are delivered into the airway at a predetermined frequency and I:E ratio. The device used in this study (manufactured by Percussionnaire Corp., Sand Point, ID) includes a unique pneumatic valve that closes the exhalation port, increasing the efficiency of delivery of the inspired breath. In order to minimize gas trapping, the cycling mechanism is interrupted and airway pressure is returned to baseline positive end expiratory pressure (PEEP) at regular intervals (usually every 2 seconds). Alveolar ventilation is controlled by varying peak inspiratory pressures and the frequency with which airway pressure is returned to baseline. Oxygenation is optimized by adjusting the mean airway pressure.

HFO involves the active injection and withdrawal of gas from the lungs. The active exhalation phase is thought to enhance gas egress, thus allowing the use of a higher frequency and lower tidal volume than with passive exhalation devices. Peak and trough pressures/volumes can be maintained close to mean lung pressure/volume, thus producing a nearly constant pressure. A fresh gas source is introduced distal to the piston. Adjustment of gas flow and resistance determine the mean airway pressure. Ventilation is provided by the delivered tidal volume with an added increment provided by the gas flow in the patient circuit.

### Experimental Design

Eighteen adult male baboons were randomized to one of four groups: group 1—control, no injury, no ventilatory support, lung lavage only (n = 3); group 2—positive pressure CON (n = 5); group 3—HFFI (n = 5); group 4—HFO (n = 5). At time 0, animals were sedated with ketamine and Valium and orally intubated; a peripheral arterial catheter and pulmonary artery catheter were inserted during local anesthesia. Baseline hemodynamic measurements, including cardiac output determined by thermodilution and arterial and venous blood gases,

Presented at the 113th Annual Scientific Session of the American Surgical Association, Baltimore, Maryland, April 1-3, 1993.

The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or reflecting the view of the Department of the Army or the Department of Defense.

Address reprint requests to William G. Cioffi, M.D., U. S. Army Institute of Surgical Research, Fort Sam Houston, TX 78234-5012.

Accepted for publication April 8, 1993.

were obtained. Pulmonary function tests were performed. A flexible bronchoscope was then passed through the right lower lobe and lavaged with two 50-cc aliquots of physiologic saline. The recovered lavage fluid was combined with an aliquot removed for cell count, with the remainder centrifuged and the supernatant decanted. A differential cell count was performed on the cell plug. The supernatant was frozen at  $-70^{\circ}\text{C}$  for later assay for total protein, total phospholipid content, and elastase content. After completion of the baseline studies, the animals were allowed to recover for 1 hour.

After repeat arterial blood gases were obtained, animals in groups 2, 3, and 4 were exposed to a moderate smoke injury using the technique previously described from this institute.<sup>10</sup> All animals exposed to smoke remained intubated after exposure and were allowed to breathe 100% oxygen spontaneously for 1 hour to reduce the carboxyhemoglobin level. Carbon monoxide levels were measured immediately before smoke exposure, immediately after smoke exposure, every 30 minutes for 2 hours, and every 4 hours for 24 hours. After 1 hour, the  $\text{FIO}_2$  was decreased to 0.21%.

Intravenous fluids consisting of D5 0.5 normal saline with 20 mEq of KCl per liter were administered at a rate of 80 cc/kg/d. Arterial lines were infused with heparinized normal saline at a rate of 2 cc/hr. All changes in fluid composition and rate of infusion were based upon hemodynamic data, urine output, and electrolyte determinations.

Ventilatory support was initiated when the  $\text{PCO}_2$  was greater than 50 torr or the  $\text{PO}_2$  was less than 50% of the baseline measurements. Animals were paralyzed with pancuronium after the ventilator was hooked up to them and then they were sedated with Valium as needed.

Arterial and venous blood gases were obtained hourly until the animal was stable and then every 6 hours or as clinically indicated. Serum electrolytes, renal function tests, and complete blood counts and platelet counts were obtained every 12 hours. Chest radiographs were obtained daily. Pulmonary function tests were performed at 0, 24, 72, and 130 hours. Bronchoalveolar lavage was performed immediately after pulmonary function testing at each time point. Alveolar lavage fluid was processed as previously described.

Animals of group 2 received positive pressure ventilation with a tidal volume of 10 mL/kg. Ventilator adjustments were made in response to blood gas determinations.  $\text{PCO}_2$  was maintained between 35 and 45 torr by adjustment of tidal volume and frequency. The  $\text{PO}_2$  was maintained between 80 and 100 torr by adjustment of PEEP and  $\text{FIO}_2$ . Initially, the PEEP was set at 3 cm of water.

HFFI was initiated in group 3 as follows. The frequency was set at 10 Hz with a 2-second inspiratory

time. The expiratory time was set to result in a return to the baseline PEEP rate of 8 breathes per minute (analogous to conventional respiratory rate). The I:E ratio of subtidal breaths was 1:1. PEEP, oscillatory in nature, was set at 5 cm of water. Peak inspiratory pressures were set at 24 cm of water. Adjustments in support were made according to blood gas determinations.  $\text{PCO}_2$  was maintained between 35 and 45 torr by adjustment of the expiratory rate and peak inspiratory pressures.  $\text{PO}_2$  was maintained between 80 and 100 torr by adjustment of peak inspiratory pressures, PEEP, and  $\text{FIO}_2$ .

HFO was accomplished in group 4 using a prototype high-frequency oscillatory ventilator (Southwest Research Institute, San Antonio, TX). Initial frequency was set at 10 Hz with an oscillatory amplitude sufficient to obtain adequate chest wall motion. Mean airway pressure was adjusted to maintain the  $\text{PO}_2$  between 80 and 100 torr. Oxygenation was optimized by adjustment of mean airway pressure and  $\text{FIO}_2$ . Ventilation was optimized by the adjustment of the oscillatory amplitude.

One hundred fifty-four hours after injury, or sooner if, in the opinion of the primary investigator, the animal was in irreversible cardiopulmonary failure, all animals were euthanized with an overdose of barbiturates. Standard necropsy was performed and sections of all organs were obtained and fixed for light and electron microscopy. The infracardiac lobe of the lung was inflated to 20 cm of water pressure, tied off, and placed in Carnoy's fixative. The right upper lobe was intratracheally instilled with 10% buffered formalin. The trachea was fixed in 10% buffered formalin in its entirety and sectioned longitudinally. A section of each of the remaining lobes was removed and frozen in liquid nitrogen.

## Data Analysis

Blood gas, blood pressure, airway pressure, and other hemodynamic data were averaged over intervals and plotted at the midpoint of each time interval. Physiologic and repetitive biochemical data were analyzed between groups using analysis of variance for repeated measures. Data were also compared at specific time points using analysis of variance. Pulmonary morphology was analyzed by three "blinded" observers using a semiquantitative technique, a panel of standards, to determine the degree of parenchymal lung injury. The technique consisted of comparing the multiple microscopic lung sections from each animal to a panel of seven standards that depicted a spectrum of pulmonary lesions, from grade 1 to grade 7 (no injury to most severe). A photomicroscope fitted with a 1X objective was used to photograph the entire cross section of the lobe. Thirty-five-millimeter negatives were photographically enlarged to yield  $4 \times 5.5$  black and white photographs.

Dist	Ava
A-1	20

Each photograph was compared with the panel and graded independently by each of the three observers. The mean of the raters' scores was calculated for each animal. Agreement among observers was determined by the Chronbach Alpha test. The RIDIT test, a nonparametric technique, was used to test for any ventilator effect on the degree of parenchymal lung injury.<sup>6,11</sup> All statistical comparisons were considered significant at a *p* value of less than 0.05.

## RESULTS

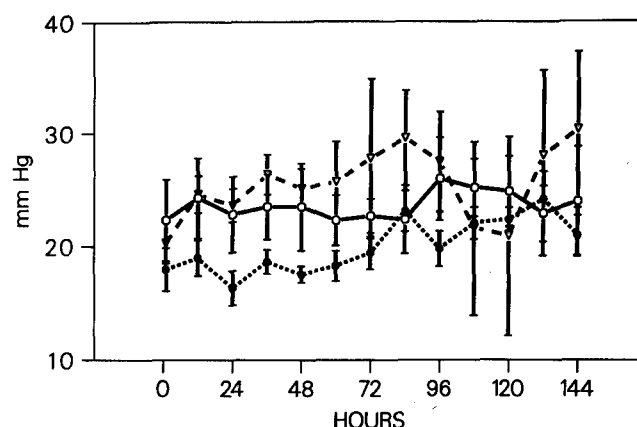
Carboxyhemoglobin concentrations immediately after injury were similar between the three smoke-injured groups (Table 1). Two animals in group 4 (HFO) did not survive the 6-day study period. All other animals were euthanized at the end of 154 hours.

### Hemodynamics

Mean systemic blood pressure did not change over time, nor was it different between groups. All animals became progressively tachycardiac over the course of the experiment, a change that was statistically different from baseline values. There were no differences between groups in the degree of tachycardia. Although cardiac output tended to vary over time, there were no significant changes over time in any one group, nor were there differences between groups at any time interval. Mean pulmonary artery pressure slightly but significantly increased over time in all groups (Fig. 1). There was no significant difference between groups in the rate of change of pulmonary artery pressure. Pulmonary artery occlusion pressures did not vary significantly between groups over time.

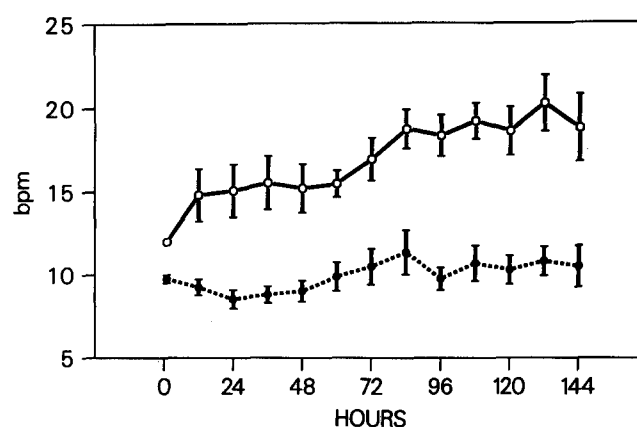
### Ventilatory Support

The fractional inspired oxygen concentration necessary to maintain oxygenation within the prescribed range significantly increased over time in all groups. The rate of change was not different between groups, and the only significant difference between groups occurred at 84 hours after injury when group 4 required significantly



**Figure 1.** Mean pulmonary artery pressure. Mean pulmonary artery pressure increased slightly but significantly over time in all animals (*p* < 0.05, analysis of variance for repeated measures). There were no intergroup differences over time. CON is represented by the solid lines with open circles, HFFI by the closed circles with the small dotted line, and HFO by the open triangles.

higher FIO<sub>2</sub> than groups 2 and 3. The respiratory rates for the CON-treated animals and the HFFI-treated animals are displayed in Figure 2. CON-treated animals required significantly greater respiratory rates than HFFI-treated animals at all time points. In addition, the IMV rate required by CON-treated animals increased significantly over time, while that required by HFFI-treated animals did not. Peak airway pressures were significantly greater in group 4 than in groups 2 and 3 (Fig. 3). Additionally, peak airway pressures significantly increased over time in group 4, but not in groups 2 or 3. PEEP requirements were different between groups 2 and 3 because of the preselected PEEP levels that were preset at the beginning of the experiment. PEEP requirements did not increase significantly over time in either of these groups.

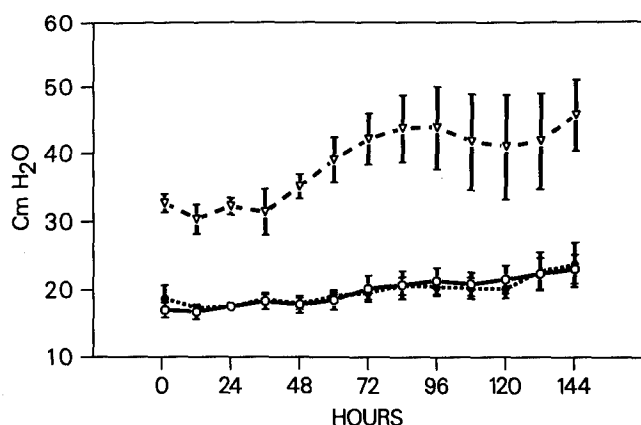


**Figure 2.** Respiratory rate. The respiratory rate of CON (solid lines, open circles) was significantly greater at all time points than the respiratory rate of HFFI (solid circles, dashed lines) (*p* < 0.05). In addition, the respiratory rate of CON increased significantly over time (*p* < 0.05).

**Table 1. CoHb LEVELS**

Group	CoHb (%)
Control	0
Conventional	42.4 ± 1.6
HFFI	44.8 ± 2.1
HFO	45.8 ± 2.1

CoHb: carboxyhemoglobin.



**Figure 3.** Peak airway pressures. Peak airway pressures significantly increased over time in HFO (open triangles, dashed lines) ( $p < 0.05$ ) and were significantly greater than CON (solid lines, open circles) and HFFI (solid circles, dashed lines) ( $p < 0.05$ ) at all time points. Peak airway pressures did not change over time in HFFI or CON.

### Arterial Blood Gas Data

Once the  $\text{FIO}_2$  was decreased from 100% in the first hour of the experiment,  $\text{PaO}_2$  did not vary between groups or over time. Arterial  $\text{PCO}_2$  was relatively constant throughout the experiment in all three groups once ventilatory support was initiated. Only at 60 and 84 hours was the  $\text{PCO}_2$  significantly higher in group 4 than the other two groups. The arterial pH tended to increase in all groups, indicating the presence of a metabolic alkalosis. No significant intergroup differences were noted except at 60 and 84 hours, the same time point at which arterial  $\text{PCO}_2$  increased in group 4. The alveolar/arterial  $\text{O}_2$  ratio did not vary significantly between the three groups over time (Fig. 4). Only at 72 and 84 hours after injury was the mean A/a  $\text{O}_2$  ratio significantly less in group 4 compared to groups 2 and 3.

### Chest Radiographs

Although there was a trend towards more atelectasis in group 4 compared to groups 2 or 3 ( $p = 0.1$ ), there were no significant intergroup differences when daily chest roentgenographs were graded for atelectasis and pneumonia.

### Pulmonary Function Tests

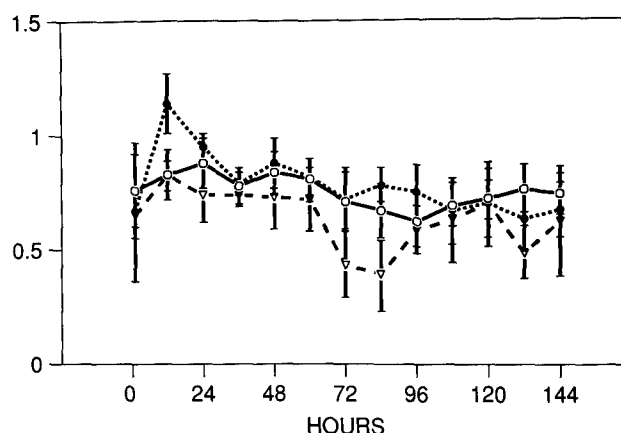
Vital capacity, inspiratory capacity, functional residual capacity, and total lung capacity all decreased significantly over time in all groups, with no intergroup differences. Effective residual volume and diffusion capacity also decreased significantly over time, but the rate of change was not different between treatment groups.

### Bronchoalveolar Lavage

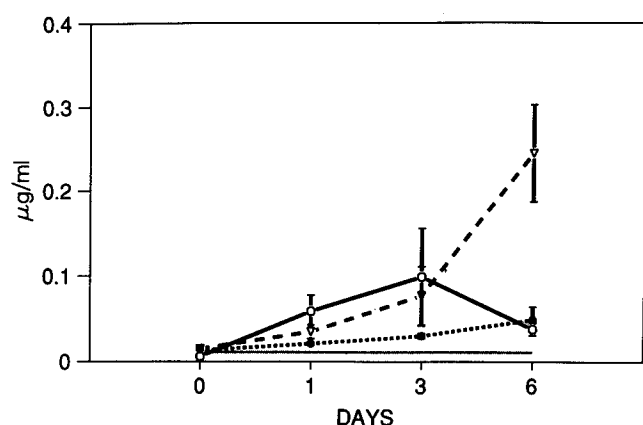
Bronchoalveolar lavage was performed before injury and after injury on days 1, 3, and 6. The lavage effluent was assayed for cell count, elastase activity, total protein, and total phosphatidylcholine content. Group 1 animals were lavaged at the same time intervals as the experimental animals. No differences between successive lavages and the measured values were noted in group 1, and therefore all control data were pooled for comparison purposes. The bronchoalveolar lavage (BAL) leukocyte counts increased significantly in groups 2, 3, and 4 over time. The rate of increase was not significantly different between groups. BAL protein content increased significantly over time in all groups. This increase in BAL protein content was sustained in both high-frequency groups, but returned to baseline in group 2 at 6 days after injury. BAL protein content was significantly higher in groups 2 and 3 compared to controls 24 hours after injury. At 6 days after injury, only groups 3 and 4 had significantly greater BAL protein levels than control animals. BAL elastase increased significantly over baseline values in groups 2 and 4 compared to controls (Fig. 5). This increase in a measurable elastase was sustained at 6 days after injury only in group 4. HFFI animals had a small but insignificant increase in elastase content of the BAL compared to control animals. Levels of phosphatidylcholine (PC) in BAL were measured at days 0, 1, and 6. There was no significant difference in BAL PC between any groups at any time point.

### Pathology

The pathologic changes in all the groups consisted of varying degrees of injury at both the airway and alveolar levels. Airway lesions included epithelial ulceration/re-



**Figure 4.** Aa  $\text{O}_2$  ratio. This ratio did not change significantly over time. At 72 and 84 hours after injury, this ratio was significantly lower in HFO (open triangles, dashed lines) than CON (solid lines, open circles) or HFFI (solid circles, dashed lines) ( $p < 0.05$ , analysis of variance).



**Figure 5.** Bronchoalveolar lavage elastase. Control values of BAL elastase content are depicted in the bottom solid line. Elastase content did not change over time in HFFI (solid circles, dashed line). BAL elastase content was significantly greater than control and HFFI in CON (open circles, solid lines) at 1 and 3 days after injury. The level returned to normal at 6 days. BAL elastase content increased significantly in HFO (open triangles, dashed lines) at 1, 3, and 6 days after injury when compared to controls and HFFI.

parative changes and mucus plugging, whereas alveolar changes were dominated by diffuse exudative alveolar damage and/or bronchopneumonia. Pulmonary parenchymal pathologic changes were scored using a panel of standards as previously described (Table 2). No significant intraobserver differences were noted. There were significant differences in pulmonary parenchymal change between groups. Using nonparametric RIDIT analysis, group 3 (HFFI) had significantly less histopathologic parenchymal damage than groups 2 CON ( $p = 0.03$ ) and 4 (HFO) ( $p = 0.0008$ ). One animal in group 3 (HFFI) was an extreme outlier, and all sections showed severe diffuse damage to a much greater extent than any other animal in any group. Excluding this animal from analysis greatly increased the differences between group 3 and groups 2 and 4 ( $p < 0.0001$ ) (Table 3).

**Table 2. HISTOLOGIC SCORES**

Grade	CON		HFFI		HFO	
	%*	CUM	%	CUM	%	CUM
1	7.8	7.8	19.9	19.9	13.3	13.3
2	25.7	33.5	36.2	56	14.9	28.2
3	25.7	59.2	18.3	74.4	21.5	49.7
4	13.3	72.5	10.6	85	16.8	66.5
5	13	85.5	7.3	92.3	15.7	82.2
6	7.9	93.4	4.5	96.8	9.5	91.7
7	6.6	100	3.2	100	8.3	100

CUM: cumulative per cent with each score.

\* Per cent of histologic sections with each score.

**Table 3. COMPARISON OF GROUPS**

Groups	RIDIT	Stat	p Value
CON > HFFI	0.6317	4.987	< 0.0000
CON < HFO	0.5368	-1.351	NS
HFFI < HFO	0.6685	-6.473	< 0.0000

NS: not significant.

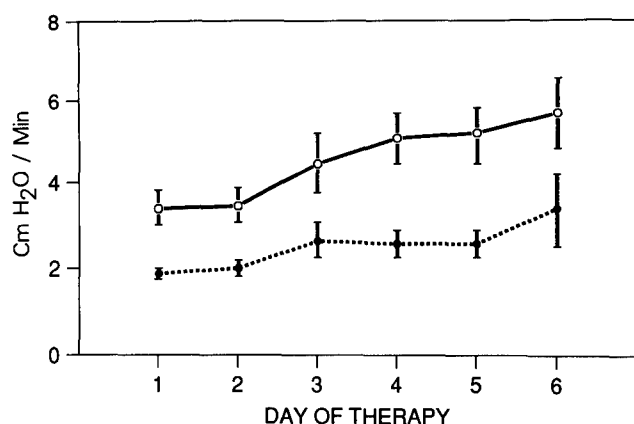
## DISCUSSION

The results of this study indicate that the prophylactic use of HFFI in smoke-injured primates led to a decrease in ventilator-induced pulmonary injury when compared to HFO and CON. That the intergroup differences in pulmonary histopathology were secondary to ventilator mode and not the original insult is supported by the identical after injury carboxyhemoglobin levels obtained immediately after similar smoke injury.

Maintenance of normal arterial blood gases was possible during the 6-day experimental period using either CON or HFFI, but not with HFO. These results are in contrast to those described with HFO in animal models of ARDS.<sup>4-6</sup> The failure of HFO in this smoke inhalation model appears to be related to the heterogeneity of the disease process and, specifically, to the airway mucosal injury. Epithelial necrosis and airway edema are typical morphologic features that lead to complete and partial airway obstruction. The resulting increase in time constants would lead to segmental overdistention given the short expiratory interval during HFO. However, with the HFFI used in these studies, two features of ventilator design/strategy would tend to overcome the limitations of imposed increased inspiratory and expiratory resistance. During the intermittent cycling pauses, lung volume is permitted to return to baseline, compensating for any increase in trapped gas volume occurring as a result of the HFV breaths. Moreover, the lung is taken through a volume cycle with HFV breaths essentially superimposed on an oscillating baseline. This allows for increased recruitment of atelectatic regions.

Comparison of the level of pulmonary support required by groups 2 and 3 revealed one difference: a significantly higher ventilatory rate at the same peak inspiratory pressures in CON-treated animals compared to HFFI-treated animals. Calculation of a barotrauma index (rate/pressure product) revealed that CON-treated animals were at significantly higher risk for pulmonary barotrauma than animals treated with HFFI (Fig. 6), despite maintenance of ventilation and oxygenation at the same target limits.

Until recently, little attention has been given to the possibility that ventilatory management may itself result



**Figure 6.** Barotrauma index. The barotrauma index was significantly greater in CON (open circles, solid lines) than HFFI (solid circles, dashed lines) at all time points.

in progressive lung injury. In the past, most of the concern expressed about possible adverse effects of ventilatory management centered upon the effect of high peak inspiratory pressures and their relationship to pneumothorax, pneumomediastinum, or pulmonary interstitial emphysema. There is now considerable evidence from animal studies that the use of high tidal volumes, resulting in high peak inspiratory pressures, can result in progressive lung injury even in mature, uninjured lungs. Positive pressure ventilation at relatively modest peak inspiratory pressures (30 cm of water) has been associated with significant alterations in lung pathology and physiology in an ovine model in which no concurrent lung insult was present.<sup>12</sup> The degree of damage in such models can be compounded by the addition of surfactant deficiency to the animal preparation. Lachmann et al. reported necrosis and inflammation of bronchiolar epithelium in a saline lavage surfactant-deficient rabbit model in which the animals were ventilated at a peak inspiratory pressure of only 20 cm of water for 2 hours.<sup>13</sup> The degree of injury was increased with marked hyaline membrane formation when the animals were ventilated at a peak inspiratory pressure of 40 cm with a reversed I:E ratio. This surfactant washout model has been previously shown to cause no major morphologic changes when the animals were allowed to breathe spontaneously, thus indicating that the changes were due to the ventilatory support modality.<sup>14</sup>

A reasonable criticism of animal studies that have demonstrated acute lung injury in normal animals subjected to high peak inspiratory pressures is the very large tidal volumes that are required to produce such injury. It is unlikely that such overdistention of the lung would occur in normal clinical practice. However, recent reports have shown that this degree of overdistention may occur in patients with acute lung injury ventilated with conventional tidal volumes because of the heterogeneity

of the insult. Gattinoni et al. have demonstrated that in patients with low compliance ARDS, only a small amount of the aerated lung contributes to ventilation.<sup>15</sup> In these patients, tidal volumes of 12 to 15 mL/kg may result in gross overdistention in the functional lung units while other poorly compliant areas remain unventilated.

Although the pathophysiologic mechanism responsible for this damage is unclear, some investigators have reported that the degree of lung damage can be partially prevented by depletion of granulocytes before ventilatory support.<sup>16</sup> In the saline lavage rabbit model, neutropenic animals ventilated at tidal volumes of 12 mL/kg showed no significant alterations in oxygenation, no change in vascular permeability, and no hyaline membrane formation or granulocytic infiltration compared to normal animals ventilated at the same tidal volume. This and other models suggest that granulocytes are at least partially responsible for the degree of ventilator-induced lung injury. In our study, there was no difference between groups in the number of granulocytes in the BAL, indicating similar recruitment of granulocytes into the lung. BAL elastase was significantly elevated in CON-treated animals on day 1 and in HFO-treated animals on day 6. In contrast, BAL elastase was never elevated in the HFFI group. Since BAL elastase is considered a marker of granulocyte activation, these data are consistent with the notion that granulocyte activation is partially dependent on mode of ventilation and may explain the lesser degree of induced lung injury in HFFI-treated animals. A similar divergence between recruitment and activation has been previously reported,<sup>17</sup> but how ventilatory mode may activate granulocytes is unknown.

Central to all studies of ventilator-induced lung injury is the requirement for alveolar overdistention. High peak inspiratory pressures in conjunction with thoraco-abdominal strapping, thus preventing lung overdistention in both rat and rabbit models, does not result in lung injury.<sup>18,19</sup> In contrast, high volume ventilation using negative pressure iron lung ventilators does result in lung injury, supporting the thesis that overdistention and not high positive peak inspiratory pressures plays a central role in causing lung injury.<sup>19</sup>

Several animal studies have suggested that the maintenance of expanded alveoli and terminal airways throughout the respiratory cycle may prevent or reduce ventilator-induced lung injury. Expansion of alveoli may be maintained by the use of PEEP, prolonged inspiratory times, or HFV.<sup>20,21</sup> The prevention of repetitive cyclical opening and closing, and overdistention of terminal airways with each ventilator breath by any of these mechanisms has been demonstrated to decrease lung injury, even when animals are ventilated at high peak inspiratory pressures.<sup>22</sup> An alternative strategy to the above

techniques would be to reduce the frequency of repeated opening and closing of airway alveolar units from a pressure below the opening pressure to one above<sup>23</sup> by a ventilatory technique such as HFFI. In our model, HFFI significantly decreased the cycles/minute in this smoke injury model (Fig. 2), while maintaining adequate CO<sub>2</sub> clearance. Because peak inspiratory pressures were the same in CON-treated and HFFI-treated animals, the maintenance of alveolar expansion at a lower respiratory rate remains a plausible explanation for our pathology findings.

These subhuman primate data support our clinical findings using HFFI in humans with smoke inhalation injury. The decrease in mortality in patients treated with HFFI compared to nonrandomized, concurrent controls and historical cohorts treated with positive pressure ventilation may be secondary to a decrease in ventilator-dependent barotrauma of an injured airway. These data strongly support the continued use of HFFI with our current strategy for the ventilatory support of patients with smoke injury to minimize additional airway injury.

## References

- Shirani K, Pruitt BA Jr, Mason AD. Influence of inhalation injury and pneumonia on burn mortality. *Ann Surg* 1987; 205(1):82-87.
- Rue LW III, Cioffi WG, McManus WF, Pruitt BA Jr. Improved survival of burn patients with inhalation injury. *Arch Surg* (in press).
- Cioffi WG, Rue LW, Graves TA, et al. Prophylactic use of high frequency ventilators in patients with inhalation injury. *Ann Surg* 1991; 213(6):575-580.
- Hamilton PP, Onayemi A, Smyth JA, et al. Comparison of conventional and high-frequency ventilation: oxygenation and lung pathology. *J Appl Physiol* 1983; 55:131-138.
- deLemos RA, Coalson JJ, Gerstmann DR, et al. Ventilatory management of infant baboons with hyaline membrane disease: the use of high frequency ventilation. *Pediatr Res* 1987; 21(6):594-602.
- Meredith KS, deLemos RA, Coalson JJ, et al. Role of lung injury in the pathogenesis of hyaline membrane disease in premature baboons. *J Appl Physiol* 1989; 66:2150-2158.
- Hurst JM, Branson RD, Davis K Jr, et al. Comparison of conventional mechanical ventilation and high-frequency ventilation: a prospective, randomized trial in patients with respiratory failure. *Ann Surg* 1990; 211(4):486-491.
- HIFI Study Group. High-frequency oscillatory ventilation compared with conventional mechanical ventilation in the treatment of respiratory failure in preterm infants. *N Engl J Med* 1989; 320(2):88-93.
- Cioffi WG, Graves TA, McManus WF, Pruitt BA Jr. High frequency percussive ventilation in patients with inhalation injury. *J Trauma* 1989; 29(3):350-354.
- Shimazu T, Ikeuchi H, Hubbard GB, et al. Smoke inhalation injury and the effect of carbon monoxide in the sheep model. *J Trauma* 1990; 30(2):170-175.
- Bross IDJ. How to use RIDIT analysis. *Biometrics* 1958; 14:18-38.
- Tsuno K, Prato P, Kolobow T. Acute lung injury from mechanical ventilation at moderately high airway pressures. *J Appl Physiol* 1990; 69(3):956-961.
- Lachmann B, Jonson B, Lindroth M, Robertson B. Modes of artificial ventilation in severe respiratory distress syndrome: lung function and morphology in rabbits after washout of alveolar surfactant. *Crit Care Med* 1982; 10:724-732.
- Lachmann B, Robertson B, Vogel J. In-vivo lung lavage as an experimental model of the respiratory distress syndrome. *Acta Anaesthesiol Scand* 1980; 24:231-236.
- Gattinoni L, Pesenti A, Avalli L, et al. Pressure-volume curve of total respiratory system in acute respiratory failure: computed tomographic study. *Am Rev Respir Dis* 1987; 136:730-736.
- Kawano T, Mori S, Cybulsky M, et al. Effect of granulocyte depletion in a ventilated surfactant-depleted lung. *J Appl Physiol* 1983; 62:27-33.
- Collins DS, Dupuis R, Sur S, et al. Neutrophil recruitment and bronchoalveolar lavage fluid elastase concentration do not correlate with one another 24 hours following segmental antigen challenge. *Am Rev Respir Dis* 1992; 145(4):A35.
- Hernandez LA, Peevy KJ, Moise AA, Parker JC. Chest wall restriction limits high airway pressure-induced lung injury in young rabbits. *J Appl Physiol* 1989; 66:2364-2368.
- Dreyfuss D, Soler P, Basset G, Saumon G. High inflation pressure pulmonary edema: respective effects of high airway pressure, high tidal volume and positive end-expiratory pressure. *Am Rev Respir Dis* 1988; 137:1159-1164.
- Webb HH, Tierney DF. Experimental pulmonary oedema due to intermittent positive pressure ventilation with high inflation pressures. Protection by positive end-expiratory pressure. *Am Rev Respir Dis* 1974; 10:556-565.
- McCulloch PR, Forkert PG, Froese AB. Lung volume maintenance prevents lung injury during high frequency oscillation in surfactant-deficient rabbits. *Am Rev Respir Dis* 1988; 137:1185-1192.
- Muscledere JG, Mullen JBM, Gan K, et al. Tidal ventilation at low airway pressures can cause pulmonary barotrauma. *Am Rev Respir Dis* 1992; 145(4):A454.
- Sykes MK. Ventilator-induced lung damage. *Acta Anaesthesiol Belg* 1988; 39 (suppl 2):43-44.

## Discussion

DR. DAVID N. HERNDON (Galveston, Texas): The authors have elegantly and convincingly demonstrated in this excellent baboon model of smoke inhalation injuries that high frequency flow ventilation, as distinguished from the more frequently studied and clinically used high frequency oscillatory ventilation technique, maintains standard physiologic endpoints as well as conventional ventilatory support with less histologic damage after 7 days of treatment.

One paradox in the data is that the protein levels in the bronchoalveolar lavage of the conventionally treated group return to normal, where they do not in either of the high frequency ventilation treated groups. White blood cell counts in bronchoalveolar lavage were not different among the three groups, leaving us to interpret the elastase data in the bronchoalveolar lavage relatively heavily.

I would like the authors to comment further on the divergence of their biochemical indices, particularly protein in the bronchoalveolar lavage, and the pathologic findings.

I think that I should emphasize the authors' findings that high frequency oscillating ventilation exacerbates morbidity in smoke inhalation injuries. This is probably due to increased air trapping with this technique.



The authors have suggested that optimally managed high frequency flow interruption respirators may improve outcome from smoke injury to the lung, but the number of patients who would have to be studied in prospective analyses to prove this would be quite large.

Finally, I would like to ask if it is possible that operator error in adapting this fairly complex technique might outweigh the potential benefits demonstrated by the study.

**DR. JOSEPH M. CIVETTA** (Miami, Florida): I wish to commend the authors for their insights that modes of mechanical ventilatory therapy influence the course of acute lung injury.

Their elegant design and careful attention to detail allowed them to show that different components can worsen a given insult in various ways. They demonstrated that high frequency flow interrupt produced the least histologic damage. They suggest that conventional ventilatory support may worsen the initial injury through granulocytic activation and production of elastase.

However, a third mechanism, high inspired oxygen tension, may also affect the course of acute lung injury. We, too, have wondered whether the improved survival we have seen in ARDS, which we had previously attributed to high levels of positive end expiratory pressure (PEEP), might have actually been due to the reflex lowering of FIO-2 whenever arterial oxygen tension rose. There are detrimental effects of high FIO-2 on both ventilation/perfusion abnormalities and the acceleration of mediator-induced lung injury.

The authors' methodology stated in the manuscript was that arterial oxygen tension would be maintained by manipulation of PEEP and FIO-2. Yet the results show that PEEP was not different among groups, but that FIO-2 did increase over time. Thus, it appears that arterial oxygen tension was maintained by increasing FIO-2, raising the possibility that this may have been a factor in the worsening of the pulmonary damage.

There were no actual numbers reported in the manuscript and I would ask the authors what FIO-2 was used, particularly in the groups that showed the most injury.

It may be that insult in combination with mechanical forces and high FIO-2 together determine the ultimate outcome. I would like the authors to comment if additional emphasis should be devoted to possible harmful effects of FIO-2 in both clinical situations and future research directions.

Again, the authors are to be congratulated for extending our understanding of the detrimental effects of therapy upon outcome, a concept not usually recognized in acute lung injury.

**DR. RICHARD L. GAMELLI** (Maywood, Illinois): I would like to compliment Dr. Cioffi and his associates on a unique and well-done study and thank them for the opportunity to review their manuscript. I address several questions to them.

The first is if they had chosen pressure limited ventilation as one of their treatment arms, what would be their expectation as to how this would have compared to their high frequency flow interrupted technique?

Second, did you determine transmural pressures with an esophageal manometer in any of your studies to give us an indication of total thoracic pressure?

Since the standard indicators of lung injury other than the

bronchoalveolar lavage elastase were not consistently different in your treatment arms but there were substantial differences morphometrically, do we need to rethink our understanding of the injury with this entity?

Do you have any information as to tissue specific proinflammatory mediators such as tumor necrosis factor alpha or as to how the mechanical process of the ventilator mode may alter these events?

A couple of practical questions: How did you control the central core temperature in your animals? Our experience in children receiving high frequency jet ventilation with significant thermal injuries has shown that maintaining their core temperature is a major problem.

Finally, if we are to employ this technology subsequently in the clinical arena, should patients be treated with high frequency flow interrupted ventilation synchronous with the diagnosis of inhalation injury? Should we gauge it by the degree of dysfunction or the severity of the insult?

I would like to thank the Society for the privilege of discussing this paper and the honor of membership.

**DR. DONALD TRUNKEY** (Portland, Oregon): I enjoyed this paper very much, Bill. A couple of questions about the high frequency flow interrupted technique. You stated that you altered the I to E ratio. What was your alteration? What was the optimal I to E ratio? What did this contribute to the overall reduction in air trapping compared to the flow interruption component of this technique?

**DR. STANLEY M. LEVENSON** (Bronx, New York): I enjoyed the paper. Dr. Cioffi, you've detailed how the photographs were looked at independently by three people who were blinded to the identifying code. Perhaps in the paper you've described, but not in the talk, how the photographs, that is the specific sections, were obtained. In other words, you have an animal that's euthanized, now you have a pair of lungs, who decided where in the lungs the 16 sections were obtained and who looked at them under the microscope to decide what areas would be photographed?

**DR. KATHRYN D. ANDERSON** (Los Angeles, California): Did you have any of these baboons receiving conventional ventilation followed by high frequency ventilation and was there an intermediate level of damage? And the clinical corollary of that is, can we use conventional ventilation and high frequency as a rescue therapy?

**DR. W. G. CIOFFI** (Fort Sam Houston, Texas): I will cover all questions in the order that they were asked.

Dr. Herndon and Dr. Gamelli both asked about the normal indexes of lung injury, i.e., alveolar lavage protein levels and white blood cell counts. We interpreted our data to indicate that the protein levels are either only an index of the original injury, or that alveolar protein levels are not as good an index of lung injury as once suspected. Most of the studies that have used protein levels to index lung injury have not used as rigid a pathologic scoring system as ours. The presence of white blood cells within the lung or in the BAL does not necessarily indicate

that those cells are activated and thus releasing either oxidants or elastase. Thus, the fact that white cells are present but do not release elastase or oxidants, indicates that the BAL white cell count is not in and of itself a good index of pulmonary injury.

Dr. Herndon asked about a prospective study. In order to do a prospective study in patients with the same decrement in mortality that we reported in our concurrent but nonrandomized study would require approximately 400 patients per arm. That is a study that could not be done in any single burn center even over a decade.

The ventilator that we used is somewhat operator dependent. However, it is not that difficult to use and our respiratory therapists are quite good at using the ventilator and training each other. I do not think there would be an enormous lag time in other units wishing to use this ventilatory mode.

Dr. Civetta asked about the inspired oxygen concentration levels and whether they were different between groups. First, they were not different between groups. Second, they did not exceed 50% in any of the three groups. The design of the experiment was that all animals would receive 100% oxygen until their carboxyhemoglobin levels returned to normal and they were then to receive room air. Oxygen concentrations were changed in order to maintain appropriate hemoglobin saturation; however, the FIO<sub>2</sub> was never raised above 50%, with the exception of the two animals in the HFO group that died. The increase in the FIO<sub>2</sub> in these animals was a perimorbid event..

Dr. Gamelli asked about pressure limited ventilation and why we didn't use that as another potential arm in this study. The first reason is that we wanted to use the ventilatory mode that is most commonly used in intensive care units, and that is volume limited ventilation. Second, we have already completed an ovine study comparing conventional ventilation and pressure limited inverse ratio ventilation and could not demonstrate a significant advantage in the early physiologic changes that occur after smoke injury. Thus, we were not interested in pursuing this idea in a much more expensive primate model. We did not place esophageal probes for measurement of interplural pressure; however, that is a good idea and should be done in the future.

We have saved BAL and plasma samples for IL-8, tumor necrosis factor, and other assays. Before performing these expensive measurements, we wanted to see what our pathology results were.

The question of whether ventilatory mode induced pulmonary damage is granulocyte mediated is complex. There is data in the literature that suggests that high tidal volume ventilation results in a granulocyte mediated pulmonary injury because when those animals are granulocyte depleted before the insult, the severity of the insult is less. That, in conjunction with our elastase data, led us to suspect that granulocytes are at least partially responsible for our observed pathology changes.

Dr. Gamelli, the temperature regulation of our animals with-

out thermal injury was not difficult. However, we used a cascade humidifier placed in line with the ventilator to warm the inspired air.

Finally, several discussants asked if this ventilator should be used prophylactically or as a salvage ventilatory mode. We reported in the *Journal of Trauma* several years ago our experience using this ventilator as salvage therapy. We were always able to reverse the physiologic pulmonary failure over a short period of time in patients who were near death and not responding to conventional ventilation. However, we did not achieve long-term salvage in any of these patients. These results led us to the prophylactic use of high frequency flow interruption in patients with inhalation injury. HFFI is instituted when the diagnosis of inhalation injury is made as long as the patients meet conventional requirements for ventilatory support. Patients were not intubated just so this ventilator could be used.

Dr. Trunkey asked about I:E ratios. When using high frequency flow interruption there are actually two I:E ratios that one must be concerned with. First is the small I:E ratio of the sub-dead space tidal volume breaths, and that is usually set as 1:1. Then there is the larger I:E ratio, if you will, of how long flow continues and how long the flow interruption period is in relation to the inspiratory flow time. We typically set that at 2:1, and do not manipulate that very much unless CO<sub>2</sub> clearance is a problem. In this animal model, CO<sub>2</sub> clearance was not difficult and thus the large I:E ratio was maintained at a 2:1 ratio.

Dr. Levenson asked who performed the lung sections and who decided what to photograph and what to study. This was all performed by one blinded pathologist who took very long longitudinal sections starting at the trachea that continued to the alveoli in the same lobe of all the animals. An average of 16 sections were taken per animal, and the entire section was then enlarged into a photomicrograph. Thus, these were not small, individual areas of the lung that were compared, but large representative lung sections. It was these photomicrographs that were then compared to the panel of standards. The three blinded observers were all pathologists at the University of Texas in San Antonio who had no previous knowledge of the animals and their ventilatory support technique. Thus, we feel we had a relatively unbiased set of observers.

Finally, Dr. Anderson asked if we had used conventional therapy first and transitioned any animal to high frequency flow interruption. We did not do that because we were not interested in pursuing this ventilatory mode as salvage therapy, but rather wanted to see if ventilatory mode after the onset of the injury could alter long-term pathology. Because that was our intent, we did not transition animals.

I think we have shown that if we initiate high frequency flow interruption immediately after smoke injury, we can significantly alter the progression of what appears to be ventilator-induced injury.